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Genetica. — *Genetic variability of alcoholdehydrogenase* (ADH) *and α-glycerophosphatodehydrogenase* (α-GPDH) *in wild and laboratory populations of* Drosophila melanogaster. Nota di GIOVANNI TRIPPA (*), ROSADELE CICCHETTI (*), MAURO SERAFINI (*), ADA LOVERRE (*) e ALDO MICHELI (*), presentata (**) dal Socio G. MONTALENTI.

RIASSUNTO. — È stato studiato il grado di polimorfismo elettroforetico di due geni, uno per l'alcooldeidrogenasi (ADH) e l'altro per l'α-glicerofosfatodeidrogenasi (α-GPDH), localizzati entrambi sul secondo cromosoma, in sette popolazioni naturali di *Drosophila melanogaster* raccolte in cantine o fuori di queste in due anni successivi. Dopo aver mantenuto la progenie delle sette popolazioni originarie come popolazioni di laboratorio (circa 10^4 individui) per circa 25–30 generazioni, sono state misurate nuovamente le frequenze alleliche dei due loci in due delle sette popolazioni di laboratorio.

I risultati ottenuti hanno permesso di valutare la ipotesi, avanzata da numerosi Autori, di un vantaggio selettivo a favore di individui portatori dell'allele Adh^4, a cui è associata un'attività enzimatica maggiore dell'allele Adh^6, e di interpretare i cambiamenti nelle frequenze geniche della ADH nelle due popolazioni di laboratorio come una conseguenza delle più grandi variazioni nelle frequenze geniche dell'α-GPDH.

INTRODUCTION

One of the most debated points in modern evolutionary genetics consists in establishing with certainty whether a genetic polymorphism is adaptive or neutral. Until very recently most of the studies on protein polymorphisms concerned the determination of allelic frequencies at one or more loci or the distribution of allelic frequencies between different populations and/or different generations of the same population in the detection of any correlations which might indicate some selection action [1-3].

Recently, an all-out effort has been made to detect the presence of a selective factor(s) and to so study its mode of action, taking into account gene frequency distribution in relation to the environment [4-6].

Numerous observations have been made in the laboratory, accentuating the environmental parameter(s) held to be the selective factor(s) in nature. In *Drosophila pseudoobscura*, heterozygotes for the octhanol-dehydrogenase gene (ODH) would, in fact, seem to be at an advantage because of the presence of octhanol in the food medium [7]. The allele $Amy^{4\cdot6}$ of the Amylase gene, which has a greater enzymatic activity *in vitro* on starch substrates than all other known alleles, markedly increases its frequency in populations

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of *Drosophila melanogaster*, maintained with starch-enriched nutrient medium [8].

Similarly, in *Drosophila melanogaster* it would seem that the greater catalytic activity of the Adh^4 allele towards ethanol could explain the more frequent occurrence of the Adh^4 allele in cellars than in other environments [9]. This has made it possible to claim that alcohol is the chief selection factor of the ADH system.

We have studied the distribution of the gene frequencies for two linked genes, *Adh* and α–*Gpdh*–1, in samples of seven wild populations collected inside or outside cellars in two successive years. After having been kept in the laboratory for at least 25 generations, two of the seven wild populations collected were also studied.

MATERIALS AND METHODS

1. *Wild populations.*

Drosophila melanogaster individuals were caught in two successive years, 1973 and 1974, in seven different sites in Southern Italy. Four of these populations were collected in cellars storing fermenting grapes, and the remaining three in the open on deposits of grape stalks.

Drosophila melanogaster males of the seven samples were assayed directly to yield the ADH and α–GPDH electrophoretic phenotype.

2. *Laboratory populations.*

The offspring of samples of the seven wild populations were kept as a laboratory population of about 10,000 individuals, with the procedure of mass-mating transfer of the adults about every 14 days. The Drosophilae were grown in 15 × 5 cm bottles containing about 30 cc. of standard nutrient medium, free from alcohol. However, only two samples of the seven populations kept were studied after about 25 generations.

3. *Electrophoresis.*

Homogenates of single individuals (about 40 μl) were subjected to horizontal discontinuous starch gel electrophoresis (1 h and 15 minutes; 4 °C; 15 V/cm; TRIS–EDTA–BORATE buffer, pH 8.0, 0.05 M in the gel and 0.5 M in the tanks). The ADH and α–GPDH phenotypes were determined for each male by using a stain mixture of 4 ml isopropanol, 4 mg NADP, 4 mg NTB, 1 mg PMS, 13 mg DL–α–glycerophosphate in 20 ml 0.1 M TRIS–HCl buffer, pH 8.5.

4. *Gene-enzyme systems studied.*

4.a. *Alcohol dehydrogenase* (ADH) *system.* Electrophoretic polymorphisms have been described for ADH in a great number of species including man [10-12].

In *Drosophila melanogaster* the ADH is controlled by a single autosomal locus (*Adh*), located on the second chromosome by Grell *et al.* [13] at 50.1. This locus turned out to be polymorphic in numerous wild populations for two electrophoretic alleles, Adh^4 and Adh^6 [14].

As far as the biochemical properties of the different isozymes are concerned, it has been found that the Adh^4 form is more active but less stable *in vitro* than the Adh^6 form [15, 16]. However, there is a wide range of variability both in the Adh^4/Adh^4 and in the Adh^6/Adh^6 strains [17]. In fact, in both homozygote strains it is possible to select lines with a greater and a lesser enzymatic activity [18]. Moreover, in that it was possible to show that different populations possess different degrees of ADH activity [19], it was concluded that there are modifiers for high and low ADH activity levels [20]. This wide variability could be of notable biological relevance for *Drosophila melanogaster* in the presence of an alcohol. Gibson [15] has in fact found that in experimental laboratory conditions the addition of alcohol to the culture medium leads to an increase in the frequency of the more active Adh^4 allele.

Recently investigations have been made into the importance of the concentration of alcohol in the culture medium as a factor capable of maintaining the high frequency of the Adh^4 allele observed in cellar populations of *Drosophila melanogaster*. Briscoe *et al.* [9] conclude that the differential mortality observed in adults of a different ADH genotype in the presence of ethanol-enriched food can contribute in keeping the high-activity Adh^4 allele more frequent in cellar populations.

4.b. *α-glycerophosphate dehydrogenase* (α–GPDH) *system.*

This system has been very thoroughly studied in *Drosophila melanogaster* from different points of view.

As far as the genetic aspects are concerned, O'Brien and MacIntyre [21] have described seven alleles of the α–glycerophosphate dehydrogenase — 1 (α–*Gpdh*–1) locus, including "null" activity mutants induced by ethylmethanosulphonate (EMS).

The map position of this locus has been set at 2–20.5.

Moreover, a series of information on the biochemical, physiological and developmental aspects of this system has been accumulated [22-24], as on the variations in enzymatic activity depending on the gene-dose of different genotypes [25, 26].

Information has also been obtained on both the seasonal fluctuations [27] and the latitudinal clines of gene frequencies indicating that the α–*Gpdh*–1^S allele is more frequent in those places with the lowest mean annual temperature [24, 28].

The interpretations from an evolutionary genetics standpoint of the almost identical results concerning this system obtained by various authors are not, however, in agreement. In fact, many Authors [24, 27, 28] are inclined to interpret their results as evidence of the adaptive significance of this poly-

morphism. Others, e.g. Leibenguth [26], on the basis of the fact that there are no great differences in the enzymatic activity associated with the α–*Gpdh*–1^{F} and α–*Gpdh*–1^{S} alleles, claim that this polymorphism probably represents a rare case of neutral alleles with no adaptive value.

RESULTS AND DISCUSSION

The most frequent ADH and α–GPDH phenotypes observed in the present study are shown in Plate I. As regards the ADH system, we found only the previously reported *Adh*^{4} and *Adh*^{6} electrophoretic alleles [14]. On the contrary, as far as the α–GPDH is concerned, in addition to the α–*Gpdh*–1^{F} and α–*Gpdh*–1^{S} electrophoretic alleles described [21], two rare variants, named α–*Gpdh*–1^{VF} and α–*Gpdh*–1^{VS}, were observed, in heterozygous combination with the fast and slow common alleles. The α–*Gpdh*–1$^{VF\ (Very\ Fast)}$ migrates more rapidly than the α–*Gpdh*–1^{F} allele and the α–*Gpdh*–1$^{VS\ (Very\ Slow)}$ more slowly than the α–*Gpdh*–1^{S} allele to the cathode. Their frequencies were pooled in the α–*Gpdh*–1$^{V\ (Variant)}$ class.

Table I gives the populations examined with the corresponding numbers and gene frequencies of ADH and α–GPDH for each of the two years.

All populations were examined for the distribution of genotypes according to the Hardy-Weinberg law in the α–*Gpdh* gene alone, the *Adh* gene being almost monomorphic. For statistical comparison the rare variants were pooled with the less frequent of the common alleles in each population. They were all in Hardy-Weinberg equilibrium.

As far as the distribution of the gene frequencies of the two systems studied is concerned, the different populations are rather homogeneous both from one year to the next, and with regard to each other. The ADH system was almost monomorphic for the *Adh*^{4} allele, with a tendency to fix this allele in cellar populations, where there is an average concentration of ethanol between 12 % and 15 %, with respect to those captured outside cellars, which develop mainly on ethanol-free substrates.

These results would not seem to support the hypothesis of selection favouring the *Adh*^{4} allele over the *Adh*^{6} allele in ethanol-rich environments. This hypothesis is based on the higher enzymatic activity level *in vitro* of the *Adh*^{4} isozyme as compared to the *Adh*^{6} allele [16] and on the observation of a differential viability of the three common ADH genotypes in the presence of artificially enriched food, at an ethanol concentration of 12.5 % [8].

Also the gene frequency in the two laboratory populations would seem to indicate an absence of this type of selection. These populations derive from the wild populations Corato 74 and Archi 74 which have an *Adh*^{6} allele frequency of 0.015 and 0.026 respectively. These values should have increased drastically during the 25–30 generations of the two populations in the presence of an alcohol-free nutrient medium, as observed by Briscoe *et al.* [9]. The gene frequencies observed, however, show fixing of the *Adh*^{4} allele.

TABLE I

Distribution of gene frequencies of ADH *and* α–GPDH *in seven wild populations (caught in two successive years,* 1973* *and* 1974**) *and two laboratory populations of* Drosophila melanogaster.

	Tot. number individuals studied	*Adh*^{4}	*Adh*^{6}	Tot. number individuals studied	α–*Gpdh*–1^F	α–*Gpdh*–1^S	α–*Gpdh*–1^V
Cellar:							
Nazzano	249*	.998	.002	299	.480	.519	.001
Castellaneta	189*	1.000	—	195	.531	.467	.002
	475**	.993	.007	516	.575	.407	.018
Otranto	213*	1.000	—	216	.528	.468	.004
	268*	.996	.004	523	.553	.442	.005
Ranna	135*	1.000	—	135	.495	.505	—
	418*	.998	.002	507	.486	.512	.002
Non–cellar:							
Corato	118*	.991	.009	118	.511	.489	—
	416**	.985	.015	496	.549	.447	.003
Archi	425*	.990	.010	485	.537	.461	.002
	881**	.974	.026	921	.503	.495	.002
Vittoria	124*	.980	.020	124	.524	.476	—
	908**	.996	.004	1.061	.516	.478	.006
Laboratory:							
Corato	151**	1.000	—	151	.679	.321	—
Archi	170**	1.000	—	170	.688	.312	—

The disappearance of the *Adh*^{6} allele cannot, however, depend only of the drift effect on the low initial frequencies of this allele in the two wild populations (0.015 and 0.026). The laboratory populations were in fact large enough (about 10^4 individuals) to prevent all the *Adh*^{6} alleles present in the original populations from being lost.

It can instead be supposed that the disappearance of the *Adh*^{6} alleles in the two laboratory populations depends on the changes in gene frequency of the *α–Gpdh–1* allele. In both populations there was observed a statistically significant increase in frequency of the allele *α–Gpdh–1*^{F}: its frequency, in fact, shifts from 0.549 (Corato) and 0.503 (Archi) to 0.679 and 0.688, respectively. It is possible that such a conspicuous increase in the frequency of the *α–Gpdh–1*^{F} allele could have influenced the gene frequency of *Adh*, despite a certain degree of linkage disequilibrium between the *Adh* and *α–Gpdh–1* loci in these two populations, the *Adh*^{6} being associated on the chromosome with the *α–Gpdh–1*^{F} allele.

The results we obtained seem to be the direct opposite of what might be expected [7, 8, 9, 16]. However, the explanation we give is fully in keeping with the hypothesis of a selective significance of the polymorphism for ADH in alcohol-rich environments, since in ethanol-free environments it can be assumed that the ADH system is no longer adaptive. It is therefore reasonable to expect that in this environment *Adh* gene frequencies depend on selection at other loci, for example the *α–Gpdh–1* locus.

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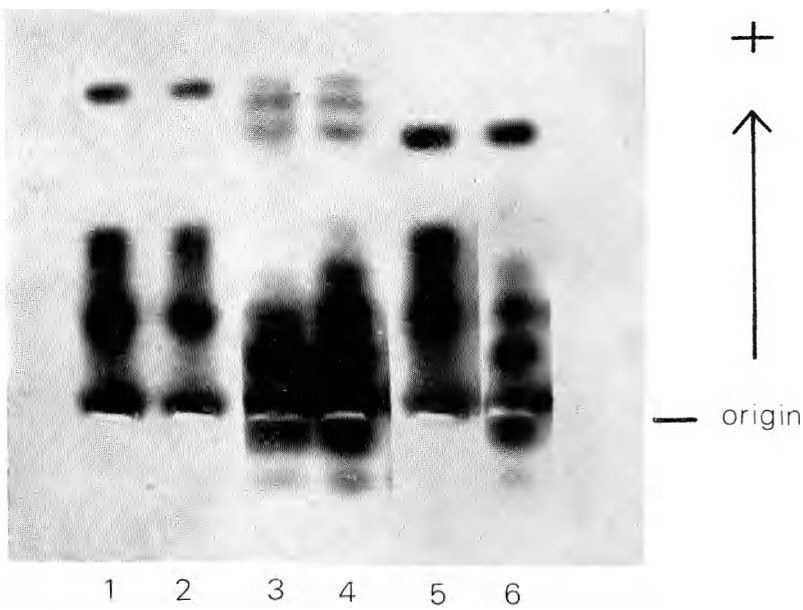

ADH (bottom) and α-GPDH (top) electrophoretic patterns of single fly homogenates. The genotypes of the single individuals are as follows: 1) and 2) Adh^4/Adh^4, α-$Gpdh$-$1^F/\alpha$-$Gpdh$-1^F; 3) and 4) Adh^4/Adh^6, α-$Gpdh$-$1^F/\alpha$-$Gpdh$-1^S; 5) Adh^4/Adh^4, α-$Gpdh$-$1^S/\alpha$-$Gpdh$-1^S and 6) Adh^4/Adh^6, α-$Gpdh$-$1^S/\alpha$-$Gpdh$-1^S.